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Although patients with HIV infection have depressed T cell function, the fact that they produce antibodies to HIV proteins (1) and even have lymphocytes capable of lysing infected target cells (2) indicates the presence of an immune response to this virus. The purpose of this contract is to devise methods to characterize that T cell response in a variety of patient populations. Once the T cell response has been defined, it should be possible to attempt to augment any response which could be effective at the elimination of virus as well as potentially dampening ineffective responses, eliminating unwanted responses. We are concerned with two major issues with regard to the interaction of T cells and HIV: 1) what make some T cells susceptible to virus?; and 2) why are some CD4 cells apparently virus resistant? Therapeutic modalities ideally would preserve T cell function while specifically eliminating the spread of virus. We have begun to investigate therapeutics with these properties with the goal of finding a safe, effective treatment.

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FOREWORD

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR56.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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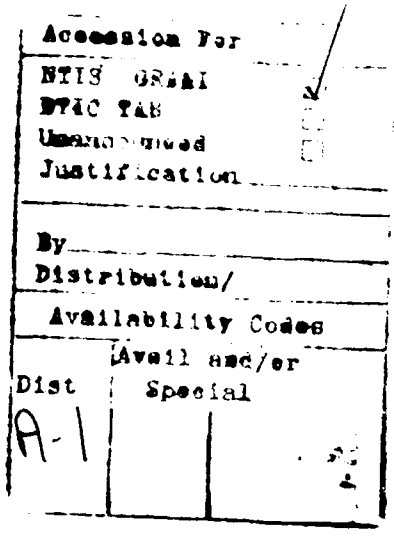

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I. STATEMENT OF THE PROBLEM

Although patients with HIV infection have depressed T cell function, the fact that they produce antibodies to HIV proteins (1) and even have lymphocytes capable of lysing infected target cells (2) indicates the presence of an immune response to this virus. The purpose of this contract is to devise methods to characterize that T cell response in a variety of patient populations. Once the T cell response has been defined, it should be possible to attempt to augment any response which could be effective at the elimination of virus as well as potentially dampening ineffective responses, eliminating unwanted responses. We are concerned with two major issues with regard to the interaction of T cells and HIV: 1) what make some T cells susceptible to virus?; and 2) why are some CD4 cells apparently virus resistant? Therapeutic modalities ideally would preserve T cell function while specifically eliminating the spread of virus. We have begun to investigate therapeutics with these properties with the goal of finding a safe, effective treatment.

II. BACKGROUND AND REVIEW OF APPROPRIATE LITERATURE

In formulating our hypothesis, we relied on the fact that patients with HIV infection have a good antibody response to the viral proteins (1) and the fact that in other human retroviruses (*e.g.*, HTLV-1) cytolytic T cells can be demonstrated (3, 4). Recently Walker and others have demonstrated the presence of lymphocytes with the capacity to lyse infected target cells in the blood of patients with HIV (2). Although previous studies of virus specific T cells had concentrated on surface glycoproteins, recent data indicates that internal proteins may be important in stimulating T cells (5). For these reasons, we are utilizing a variety of proteins as stimuli. The recent use of peptides rather than whole proteins as stimuli for target for CTL (6) has broadened our horizons of potential antigens which can be evaluated.

Recent publications document that HIV-1 specific T cell clones can be derived from AIDS patients (7). However, despite the presence of such clones, the disease progresses. Therefore, either these clones are not effective at eliminating the virus or they themselves are sensitive to the virus. Thus, we are currently investigating ways by which clones which are not susceptible to infection may be induced. In the last few years, a group of $CD4^-$ T cells have been described. These cells which are like conventional $CD4^+$ or $CD8^+$ T cells are $CD3^+$ and have a receptor characterized by a two chain structure. However, instead of having the $\alpha\beta$ dimer found in 90% of T cells, this subset has an alternative structure referred to as the $\gamma\delta$ receptor (8). The importance of such cells has not been defined.

It is conceivable that CD4- T cells will be resistant to infection with HIV-1 (since HIV-1 binds to the target cells by utilizing CD4 as a receptor). While the presence of CD4 seems to be sufficient for infection to occur in certain circumstances, we have found situations in both human and murine cells where the presence of CD4 is not sufficient for HIV-1 infection (see Results section). This has led us to investigate differences between CD4 cells which might result in some being resistant to infection. From a cellular standpoint, elimination of HIV-1 could be achieved by: 1) toxins which would specifically eliminate infected cells; or 2) agents which could inactivate the virus or prevent its spread. We have examined two agents, one from each category, and report on their *in vitro* activity.

III. RATIONALE USED IN CURRENT STUDY

Our eventual goal (as stated in the contract proposal) will be to augment protective immunity. In order to obtain such reagents, however, it is necessary to find T cell clones which will: 1) protect against virus; and 2) resist virus infection. To do this, we have had to develop methods to 1) support the growth of T cells from HIV positive patients and 2) define what antigens will be stimulated.

In addition to defining the presence of virus specific T cell clones as others have done (7), we are approaching the question of how a T cell may be resistant to viral infection. We are doing this by utilizing $CD4^-$ tumor cells (both human and mouse) and then transfecting them with CD4. By eliminating virus and virus-infected cells, we have investigated agents which may be efficacious in the treatment of HIV-1 infection.

IV. EXPERIMENTAL METHODS

In order to support the growth of lymphocytes from HIV positive patients, we have used the following protocol.

Isolation of Mononuclear Peripheral Blood Cells and Proliferation Assays

Human mononuclear peripheral blood cells (PBL) were isolated under sterile conditions from 60-100 ml heparinized peripheral blood by ficoll-hypaque density gradient centrifugation. Thirty ml aliquots of blood were layered over 15 ml of lymphocyte separation (LSM) medium (Litton Bionetics, Kensington, MD). After centrifugation, cells at the interface were collected, and washed twice in a culture medium consisting of RPMI 1640 medium (Gibco, Grand Island, New York) supplemented with 5% human AB serum (MA Bioproducts, Walkersville, MD), 2mM glutamine (Gibco), and 100 U/ml penicillin-streptomycin (Gibco).

Generation of $CD4^-$, $CD8^-$ ($\gamma\delta^+$) T cells

PBMCs from blood drawn from a healthy PPD-reactive individual were separated by sodium diatrizoate/Ficoll centrifugation and then cultured at 2×10^6 ml^{-1} in complete medium (RPMI 1640 plus 15% autologous serum plus 100 μg ml^{-1} streptomycin and 100 U ml^{-1} penicillin) together with 10 μg ml^{-1} PPD (Statens Seruminstitut, Copenhagen) in a 25-cm^3 flask (Costar) and incubated at 37°C in a 5% CO_2/95% air. On day 6, viable cells were separated by density-gradient centrifugation and recultured with fresh autologous irradiated (4,000 rad) PBMC feeders and PPD. On day 9, viable cells were recovered and $CD4^-$ and $CD8^+$ cells depleted. Briefly, the cells were incubated with supernatants from OKT4 and OKT8 hybridomas for 30 min at 4°C and then washed and further incubated with goat-anti-mouse-antibody coated latex-polymer beads with a magnetic core (Advanced Magnetics) at 37°C, on a gyratory shaker for 20 min. Subsequently, $CD4^+$ and $CD8^+$ cells bound to the beads were removed by a magnet (BioMag Separator, Advanced Magnetics). The remaining cells were washed and cultured with irradiated autologous feeders (1×10^6 ml^{-1}), PPD, and 10% T-cell growth-factor-containing media (Lymphocult, Biotest AG, Frankfurt). This cycle was repeated every 7 days and the expanded cells were depleted of $CD4^+$ and $CD8^+$ cells repeatedly, together with an anti-$\alpha\beta$ (BMA 031) antibody for

depletion of αβ lymphocytes. Phenotypic marker analysis was determined by standard double-color FACS. The monoclonal antibodies used were anti-Leu-4-PE (Becton) for the CD3 marker, BMA 031 for αβ, and TCR δ1 for γδ. For the unlabelled antibodies, a second layer of labelled goat-anti-mouse-FITC (Becton) was applied before FACS analysis. Controls were run with isotype-matched antibodies.

Production and Infection of CD4 Expressing Human Tumor Cells

HSB2, a $CD4^-$ human T cell tumor, was cocultured with the retroviral producer lines MNST4 DAMP (9) and MNCT4PI DAMP in order to confer expression of CD4 and CD4PI, respectively. The protein encoded by this hybrid gene has authentic CD4 sequence up to amino acid 371 and, following a five amino acid insertion shown in single letter code, the authentic sequence of the GPI-anchored form of LFA-3 from amino acid 210. The hybrid gene consists of CD4 cDNA sequence through the Fnu4HI site at 1257 (10) where a BamHI linker was inserted. It then continues, to the end of LFA-3, through a BamHI linker inserted in the NspBII site at 636 (11). These producer lines are obtained by transfection of the amphotropic helper line DAMP with defective proviral vectors carrying neomycin resistance and the gene in question, as has been previously described (9, 12, 13). The HSB2 infectants were selected in the antibiotic G418 and screened for expression of CD4(PI) by immunofluorescent staining with OKT4. $CD4^+$ lines chosen for initial experiments were subsequently cloned by limiting dilution. Cells were stained with OKT4 hybridoma supernatant and FITC (fluorescein isothiocyanate) goat anti-mouse IgG $(Fab')_2$ (FITC GAM) (Tago) or FITC GAM alone and fixed in 1% paraformaldehyde. The clones derived after transfection with CD4PI (e.g., P17.13) had a CD4 molecule whose surface expression was diminished by incubating with phosphatidyl inositol specific phospholipase (PIPLC). Other clones derived from conventional CD4 constructs (*e.g.*, CD410) were membrane anchored and therefore expression of CD4 was not susceptible to cleavage by PIPLC.

Production and Infection of CD4 Expressing Murine Tumor Cells

cDNAs encoding the human CD4 protein were introduced into two murine T cell hybridomas, By155.16 and 5D5.63 by cocultivating the hybridomas with the amphotropic producer line, MNST4 DAMP (9). By155.16 is the product of a C57BL/6 splenic T cell fused with the thymoma BW5147. 5D5.63 is an autoreactive T cell hybridoma generated by fusing cultured lymph node cells from an autoimmune MRL/Mp-lpr/lpr mouse with BW5147. Expression of human CD4 proteins was demonstrated in all cells by staining with monoclonal anti-OKT4, OKT4a, and Leu 3a antibodies.

Examination of *In Vitro* Cultures for Efficacy of Anti-HIV Drugs

T cells or tumors were infected with HIV-1 (MN or III_B strains) by incubation (for 1 hr at 37°C) with H9 culture supernatants. After infection, cells were washed three times and incubated in medium alone overnight prior to their addition to uninfected CD4+ T cells (derived from peripheral blood "leukopacks" and depleted of NK cells and CD8+ cells as described above) or tumors (H9 cells). Compounds of interest were added at the time of mixing. In the case of IL-2 toxin, the drug was added on days 1 and 3 and in the case of CPFs, the compounds were added at a dose of 50 μg/ml every three days.

Infection was monitored by production of: 1) p24 in supernatants; 2) HIV-1 specific RNA assessed by RNA-RNA hybridization; and 3) immunoprecipitation of ^{35}S methionine labelled HIV-1 infected cells with anti-HIV antisera.

RESULTS

A. Addition of interferon-γ (*in vitro*) enhances lymphocyte function of cells from patients with AIDS

1. The effects of rIFN-γ and rIFN-α on the proliferative responses of lymphocytes isolated from patients with Kaposi's Sarcoma (KS)

   The *in vitro* addition of IFN-γ but not -α to the PBL isolated from patients with AIDS-KS resulted in augmented proliferative response to the lectin PHA ($p < 0.01$; Wilcoxin's and Friedman's test). The proliferative response to the viral antigens HSV and CMV, and to *Candida albicans*, was increased in the AIDS-KS cultures supplemented with IFN-γ ($p<0.01$; Neuman Keuls' test). IFN-α, on the other hand, inhibited the responsiveness of the AIDS-KS lymphocytes to PHA ($p<0.05$; Wilcoxin's and Friedman's test) and to the microbial antigens HSV, CMV, and *Candida albicans* ($p<0.01$; Neuman Keuls' test) (14).

   Neither rIFN-γ nor rIFN-α were directly mitogenic. In the absence of an additional mitogenic stimulus (lectin or antigen), the cytokines were without effect on the proliferation of the lymphocytes ($p>0.05$, NS; Neuman Keuls' test (14).

2. The effects of IFN-γ and IFN-α on the proliferative responses of lymphocytes isolated from patients with a history of opportunistic infection

   Interferon-γ significantly increased the response to PHA in cultures established form the AIDS-OI patients ($p<0.01$; Wilcoxin's and Friedman's test). Whereas IFN-α enhanced the proliferation to PHA of the lymphocytes isolated from the AIDS-OI patients, IFN-α was without effect on the lectin response ($p>0.05$, NS; Wilcoxin's and Friedman's test) (14).

   In the cultures of lymphocytes derived from the AIDS-OI patients, neither IFN-γ nor IFN-α were found to have significant effects on the proliferative response to the microbial antigens ($p>0.05$, NS; Neuman Keuls' test). The effects of the cytokines at doses of 5, 500, and 50,000 U/ml were equivalent in the AIDS-OI cultures stimulated with the microbes. Interferon-γ, at doses of 5 to 50,000 U/ml, failed to stimulate the proliferative response to antigens of the lymphocytes isolated from the AIDS-OI patients (14).

B. Administration of Interferon-γ to patients resulted in decreases in levels of HIV p24 antigen in the serum.

As part of a trial of IFN-γ in patients with AIDS, we measured serial p24 values in patients receiving IFN-γ. These results (15) suggest that IFN-γ may have a role as an adjuvant agent in the treatment of AIDS.

C. CD4 expressing T cell clones may not be infectable with HIV-1

1. The ability to infect a human T cell clone is dependent not only on its ability to express CD4.

   Monoclonal populations of the HSB tumor transfected with CD4 we restained for expression of CD4. CD4 expressing cells were divided into high expressors (clone M23 is shown in Figure 1) or low expressors (clone P17.13 is shown in Figure 1).

   When infected with HIV-1, the "low" expressing clones more easily infected with HIV-1 with expression of viral proteins occurring within a few days (Figure 2).

   High expressing clones were not infectable even with extremely high titers of virus. In order to investigate whether the level of expression of CD4 was responsible for the lack of infection, cells were preincubated with a non-blocking monoclonal antibody to CD4 ($OKT4_c$) and goat anti-mouse antibody. Such preincubated cells were capable of infection with HIV. While 10.28 and M23 could not be infected without "modulation of CD4," both expressed p24 antigens within six days of infection when the incubation was carried out with CD4 antibodies and a goat anti-mouse second antibody.

2. The CD4 molecule does not need a membrane anchor to serve as a receptor for HIV-1.

   While HSB transfectants with low expression of CD4 may be infected, a membrane spanning region is not necessary in order to infection to occur. Substitution of the C terminus of CD4 with the cDNA encoding the C terminus of LFA-3 resulted in a molecule containing the HIV-1 binding site but not the transmembrane sequences. A clone of cells expressing this CD4 "variant" molecule was as easily infected as a clone expressing the transmembrane CD4 (16).

D. Antigen specific CD4 negative human T cell clones can be derived from peripheral blood

In a study aimed at elucidating the participation of $TCR_{\gamma\delta}^+$ T cells in the immune response to antigen, we cultured peripheral blood mononuclear cells (PBMC) from an immune individual with PPD for six days and analyzed the change in the percentage of TCR $_{\gamma\delta}^+$ lymphocytes by flow cytometry after staining with antibody directed at the δ chain of the TCR $_{\gamma\delta}$ heterodimer. The results of such studies revealed a fourfold increase from the baseline of 4% $TCR_{\gamma\delta}^+$ of the $CD3^+$ T cells in the unstimulated PBMC to 16% after stimulation with PPD (data not shown). This expansion of $CD3^+TCR_{\gamma\delta}^+$ lymphocytes may indicate either an antigen specific stimulation of these lymphocytes, or a non-specific bystander and passive expansion of these cells in response to growth factors produced by the predominant $TCR_{\alpha\beta}^+$ cells. We therefore designed experiments to find out if there is an antigen specific expansion of $TCR_{\gamma\delta}^+$ T cells.

In order to isolate antigen reactive $TCR_{\gamma\delta}^+$ T cells, we raised a PPD reactive line by stimulating PBMC with PPD and by restimulation with antigen and fresh feeders for an additional three day period. Subsequently, we enriched for $TCR_{\gamma\delta}^+$ cells by negatively depleting $CD4^+$ and $CD8^+$ lymphocytes by incubating the line with anti-

CD4 and anti-CD8 monoclonal antibodies and removing the $CD4^+$ and $CD8^+$ cells with anti-mouse immunoglobulin coated latex beads. Such CD4 and CD8 depleted cells were then expanded by incubating with fresh antigen presenting cells, PPD and T cell growth factor containing media. Further serial depletion of this line with anti-CD4 and anti-CD8 antibody followed by an anti-$TCR_{\alpha\beta}$ antibody resulted in a homogeneous and stable $TCR_{\gamma\delta}{}^+/_{\alpha\beta}{}^-CD3^+CD4^-CD8^-$ line (GD line) as revealed by double staining with anti-CD3 antibody and the appropriate anti-TCR antibodies or double staining with anti-CD4 and anti-CD8 antibodies. Furthermore, immunoprecipitation of ^{125}I-labelled GD cells with anti-TCRγ1 and $TCR_{c\gamma}$ antibody revealed a 40 kDa TCRδ chain and a 55kDa TCRγ chain, respectively (17). The δ subunit showed a marked shift in mobility under reducing and non-reducing conditions consistent with previous reports for $TCR_{\gamma\delta}{}^+$ T cells. These cells were also shown by flow cytometry to express CD2 (sheep red blood cell receptor), UCHL-1, the activation antigens HLA-DR and the IL-2 receptor, but lacked CD45R, and CD16 (Fcγ receptor and a natural killer cell marker) antigens. The purity of the line was functionally confirmed by incubating the line with monoclonal antibodies directed at components of the TCR complex, namely, CD3, and δ chain and the $\alpha\beta$ complex in the presence of plastic adherent APC. The results of such studies (data not shown) revealed the line to proliferate in response to either anti-TCRδ1 or anti-CD3 but not to anti-$TCR_{\alpha\beta}$ antibodies providing further evidence that the GD line is composed only of $TCR_{\gamma\delta}{}^+$ cells (17).

The specific functional capability of the GD line was tested by a proliferative assay against PPD and an irrelevant antigen. For such studies, GD cells were cultured together with autologous APC in the presence of either PPD, formalin inactivated candida antigen, or media alone. The results of such experiments showed a marked proliferative response to PPD but essentially no response to candida antigen, reflecting the specificity of these $TCR_{\gamma\delta}{}^+$ lymphocytes to PPD. These findings, therefore, provide evidence that $TCR_{\gamma\delta}{}^+$ T cells do indeed recognize antigen specifically as do $TCR_{\alpha\beta}{}^+$ lymphocytes. Since PPD is a crude culture filtrate of Mycobacterium bovis strain BCG and contains several tuberculoproteins, we obtained a well-characterized and purified mycobacterial antigen in order to better assess antigen specificity. We therefore tested the response of the GD line to purified recombinant 65 kD HSP cloned from BCG. The results of such studies demonstrated the line to have a vigorous response to this antigen, indicating the 65 kDa mycobacterial HSP to be an important inducer of $TCR_{\gamma\delta}{}^+$ T cells (17, 18).

E. A chimeric toxin moleucule is able to specifically eliminate HIV-1 infected cells.

1. HIV-1 infection stimulates expression of IL-2 receptors on T cells

CD4+ cells (from the peripheral blood of HIV-1 negative blood donors) were prepared by negative selection of B cells, macrophages, NK cells and CD8+ T cells (see Experimental Methods, above).

Ten million cells were incubated with $HTLV\text{-}III_B$ (derived from filtered supernatants of infected H9 cells, AIDS Research and Reference Reagent Program) for 1 hr at a multiplicity of infection of 10 (determined by limiting dilution of H9 cells) followed by an assessment of p24 expression by ELISA (Abbott) 1 wk after infection.

Cells were cultured at a density of $2x10^6$ cells/ml in RPMI 1640 with 10% bovine calf serum at 37°C in 5% CO_2 without any added lymphokines. The presence of IL-2 receptor was assessed by serial fluorescence analysis of cells using the TAC antibody (gift of Dr. T. Waldmann) (Figure 3). Cell viability was 100% until day 6 at which time it fell to approximately 80% in both groups. The percentages are means of three identical experiments.

2. DAB_{486} IL-2 selectively kills HIV-1 infected T cells

DAB_{486} IL-2 is a chimeric toxin molecule produced by replacing the native diphtheria toxin binding domain with human interleukin-2 (IL-2) sequences (19). This molecule, described by Williams et al. (19) is selectively toxic for T cell (or tumor cellss) which express the IL-2 receptor (20). CD4+ cells (obtained as described in Experimental Methods) were cultured at $2x10^6$/ml in 8 ml in 6 well tissue culture plates in RPMI 1640 and 10% BCS supplemented with Lymphocult-T (Boehringer-Mannheim at 10 units/ml or approximately 10^{-9} M IL-2 concentration). Cells were infected as described above. 10^{-7} or 10^{-8} M DAB_{486} IL-2 was added on days 1 and 3 post infection. Cells were split twice weekly and viability determined on the basis of trypan blue exclusion. Uninfected cells were >90% viable at two weeks and infected cells were >75% viable at two weeks without DAB_{486} IL-2, (data not shown). Percent viability is based on three experiments and a representative experiment is illustrated (Figure 4).

3. DAB_{486} IL-2 treated T cell cultures do not produce HIV-1 proteins

T cells, infected as described above, were incubated overninght in 10% BCS and washed three times prior to addition to uninfected T cells from the same donor. One million infected T cells were mixed with $10x10^6$ uninfected T cells and cultured in 10^{-9} M IL-2 (Lymphocult T, Boehringer-Mannheim) with or without DAB_{486} IL-2 (10^{-8} M) at 10^6 cells/ml in six well cluster dishes. Cells were pelleted, washed twice and resuspended every two days in IL-2 containing medium. DAB_{486} IL-2 was added on days 1 and 3 and washed out 24 hrs later. Uninfected cell cultures were treated in an identical manner.

Two weeks after infection, cells were labelled overnight with ^{35}S methionine-free medium (Figure 5). Immunoprecipitation was performed with anti-HIV globulin (AIDS Reference and Reagent Program, catalog number 192, left panel) or the framework anti-class I MHC antibody W6/32 (right panel). Lane A: infected T cells alone; Lane B: uninfected T cells; Lane C: 10^6 infected cells mixed with $10X10^6$ uninfected cells; Lane D: identical to Lane C but 10^{-8} M DAB_{486} IL-2 was added on days 1 and 3 post infection.

4. DAB_{486} IL-2 prevents cell-to-cell infection by HIV-1

T cells were prepared and maintained as described above. Cells were split 2-3x/week and adjusted to a concentration of $2x10^6$/ml. Supernatants were analyzed by ELISA for the presence of p24 (Abbott) 48 hours after splitting the cells. Hatched bars represent the supernatants of 10 x 10^6 HIV-1 infected T cells. Stippled bars represent supernatants cultures containing 10^6 infected cells mixed with 10 X 10^6 uninfected T cells. Open bars represent supernatants of the same mixtures of cells which were treated with DAB_{486} IL-2 on days 1 and 3 post infection. Solid bars represent the

supernatants of uninfected cells. No p24 was detected in either of these two groups (Figure 6).

F. A dipeptide molecule is able to prevent HIV-1 binding and spread of infection.

1. A dipeptide molecule binds to gp120 and inhibits HIV-1 binding

Although soluble CD4 has been demonstrated *in vitro* to inhibit HIV-1-CD4 interactions, treatment of patients with a complex protein is likely to be difficult because of its immunogenicity and a limit to the concentrations which can be reasonably achieved. A mutational analysis had indicated that a particular phenylalanine residue of the N terminus of CD4 was important in CD4-gp120 binding. For this reason, we tested a series of phenylalanine containing molecules for their ability to inhibit gp120 binding to CD4. One particular dipeptide, consisting of a prolyphenylalanine with an NH2-terminal carbomethoxycarbonyl moiety and a COOH-terminal benzyl ester (termed CPF), had activity in inhibiting gp120 binding to CD4 (21). The D,D entaminer (CPF D,D) had greatest effect on inhibiting binding activity.

2. The gp120 binding compound is capable of restoring CD4-class II conjugate formation which is inhibited by gp120

One of the mechanisms by which HIV-1 appears to inhibit T cell function is through the direct interaction of the gp120 molecule which inhibits T cell function by binding CD4. It was therefore of considerable interest that CPF D,D restored the ability of CD4- T cells to interact with class II molecules despite the addition of soluble gp120 (21).

3. Addition of CPF D,D to virus inhibited production of HIV 1 proteins by susceptible cells.

In a series of experiments with either T cells or susceptible tumor cells, we were able to demonstrate that a 1 hr preincubation with CPF D,D markedly limited the ability of the virus to lead to production of HIV-1 RNA or protein (21).

Incubation with CPF at a dose of 80 mg/ml inhibited any evidence of active infection. Thus, we hypothesize that by binding to gp120 the dipeptide either: 1) prevents infection by inhibiting the gp120-CD4 interaction; or 2) affects the stability of the virus in a manner which inhibits infectivity.

D. Addition of CPF prevents cell-to-cell spread of virus.

At a dose which can be tolerated by growing cells (50 mg/ml), the addition of CPF D,D to cell cultures consisting of infected T cells and uninfected tumor cells resulted in inhibition of HIV-1 infection indicating that CPF can prevent cell-to-cell spread of HIV-1 (21).

DISCUSSION AND CONCLUSIONS

The long term goal of these studies is to find ways of amplifying the human T cell response to HIV-1 in such a manner that it is useful in protecting against the development of AIDS.

In approaching this problem, two obvious problems present themselves. The first problem relates to the fact that patients with HIV-1 infection have poor T cell responses to all specific antigens. We have approached this problem by using an immunomodulator: IFN-γ (which is also an anti-viral agent) to enhance antigen specific immune responses. These studies (concluded in the first year of the contract) are described in our publication "Recombinant Human IFN-γ enhances *in vitro* activation of lymphocytes isolated from patients with AIDS" (14).

The problem with which we have dealt in this portion of the contract concerns the development of clones and their resistance to the lytic effects of the HIV-1 virus. We have used two approaches to investigate this problem.

Taking advantage of the fact that HIV-1 infects cells via CD4, we have established antigen specific T cells which are CD4-. We have found that these cells are $CD3^+$ and bear the $\gamma\delta$ T cell receptor. Whether such cells (which might be anticipated to be resistant to HIV-1) will be susceptible to the virus is currently under study. If they are resistant, we will concentrate on isolating such $\gamma\delta$ clones (in preference to $\alpha\beta$ clones) because of their ability to withstand viral infection.

In a murine model of infection, we have demonstrated that some virus specific T cell clones may be resistant to lysis by a virus despite the presence of surface receptors (22). It will be essential in future work to determine whether we can isolate such clones from patients with HIV-1.

Another strategy to develop resistant cells is to alter the CD4 molecule in a manner which prevents HIV-1 infection. We have demonstrated that the CD4 molecule does not require a membrane anchor in order to serve as a virus receptor (16).

The converse of this statement is shown in the other experiment we have performed using transfected tumors. In these experiments, a high level of CD4 expression negatively affects the ability of HIV-1 to infect the cell. A reduction in CD4 expressing (activated by antibody induced modulation) resulted in infection. These data and the results obtained with the murine clones both suggest that internalization of a CD4-HIV-1 complex may be essential for HIV-1 infection.

A cell line which produces a high level of CD4 is not susceptible to infection. These data suggest that immunological or pharmacologic manipulations which increase CD4 expression and decrease CD4 internalization may decrease HIV infection of T cells. Since the major reservoir of the virus in AIDS patients appears to be circulating $CD4^+$ T cells, these manipulations may be of therapeutic importance.

We have utilized two further approaches to develop a therapy which will specifically eliminate infected cells while preserving the ability of other T cells to function appropriately.

In the first approach, we utilized a chimeric molecule which selectively bound to infected cells. We were able to demonstrate that we could "pick out" the HIV-1 infected cells without affecting the function of the remaining T cells.

In another series of experiments, we demonstrated that a dipeptide compound with gp120 binding activity could: 1) prevent infection by HIV; and 2) allow the selective growth of uninfected T cells and tumor cells. Since the presence of the dipeptide compound (CPF) by itself was able to reverse HIV-1 gp120 induced inhibition of T cell activity, this agent has great potential as a therapeutic.

On the basis of these results, we conclude that agents such as IFN-γ which have antiviral activity as well as T cell modulating activity may have a role combination therapy. In addition, two new approaches to selective elimination of HIV-1 infected cells (using a chimeric toxin molecule), or using a gp120 binding compound may allow for selective elimination of those T cells which are HIV-1 infected and eventually control progression of the viral infection.

FIGURE LEGENDS

Figure 1. CD4 staining of high expressor (CD4M) and low expressor (P17.13) transfectants of HSB (parent line).

Figure 2. Low expressor HSB transfectants are susceptible to HIV-1 infection.

Figure 3. Exposure to HIV-1 stimulates expression of IL-2 receptor on T cells.

Figure 4. DAB_{486} IL-2 selectively kills HIV-1 infected T cells.

Figure 5. Addition of DAB_{486} IL-2 eliminates HIV-1 replication in monocytes.

Figure 6. DAB_{486} IL-2 treated T cell cultures do not produce HIV-1 proteins.

FIGURE 1

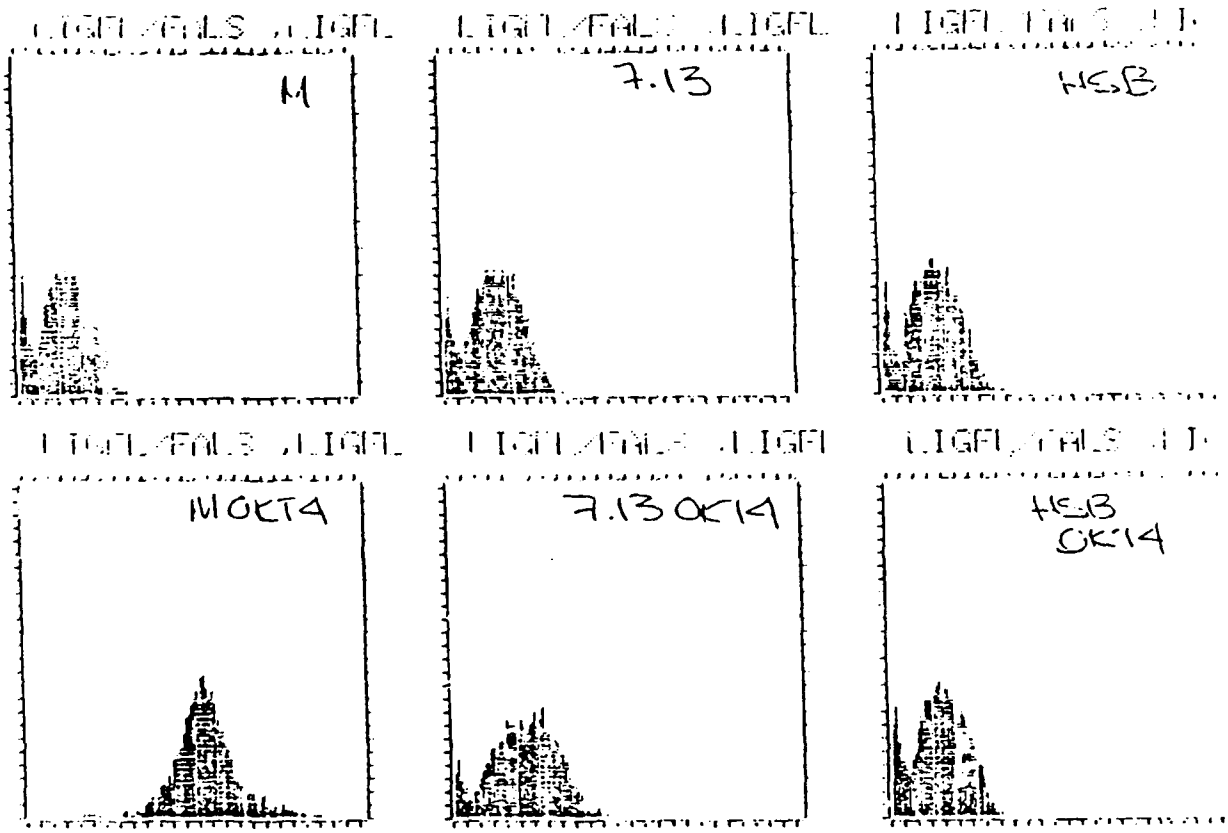


FIGURE 2

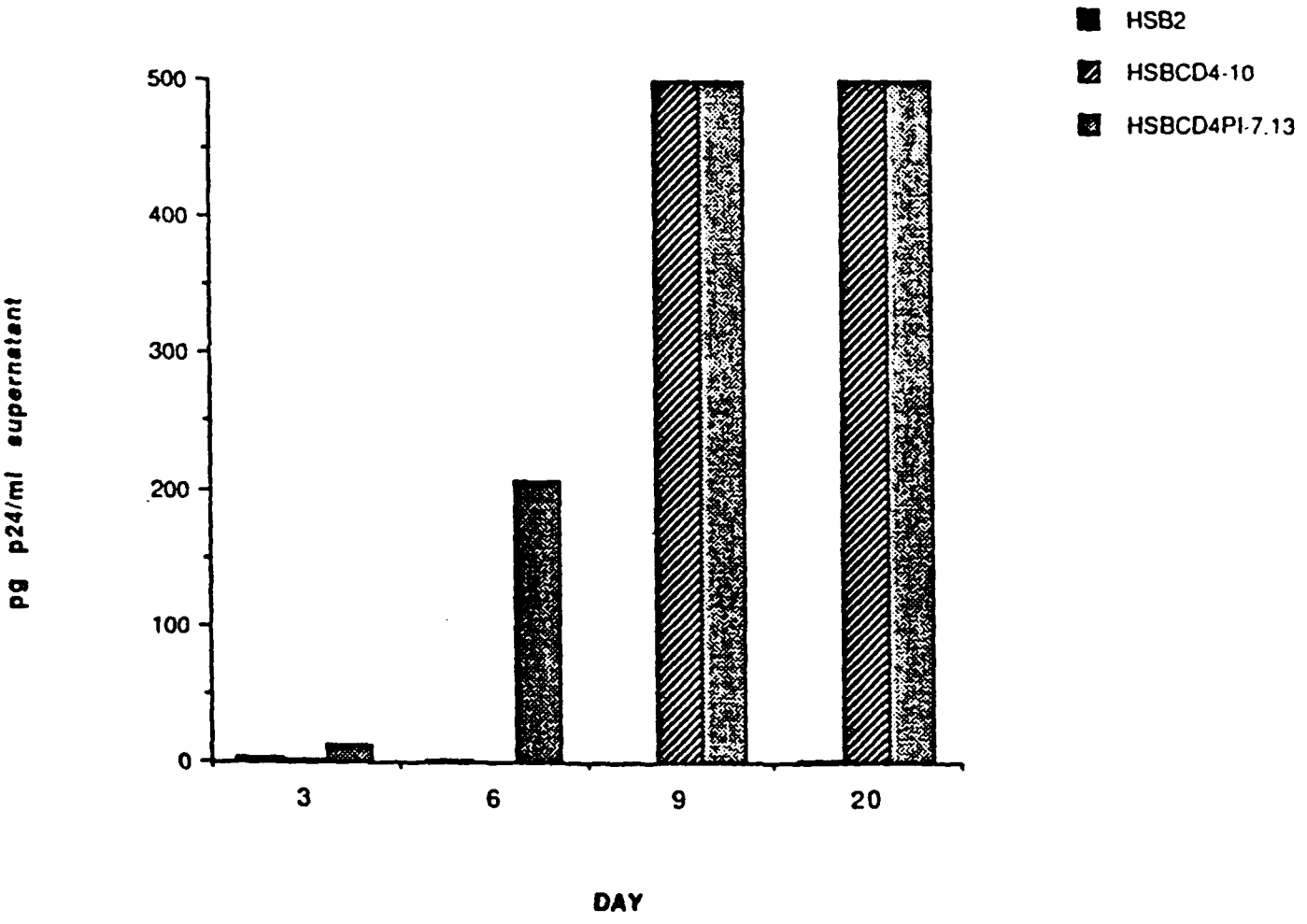

HSB2
HSBCD4-10
HSBCD4PI-7.13
500
400
300
200
100
0
pg p24/ml supernatant
3
6
9
20
DAY

FIGURE 3

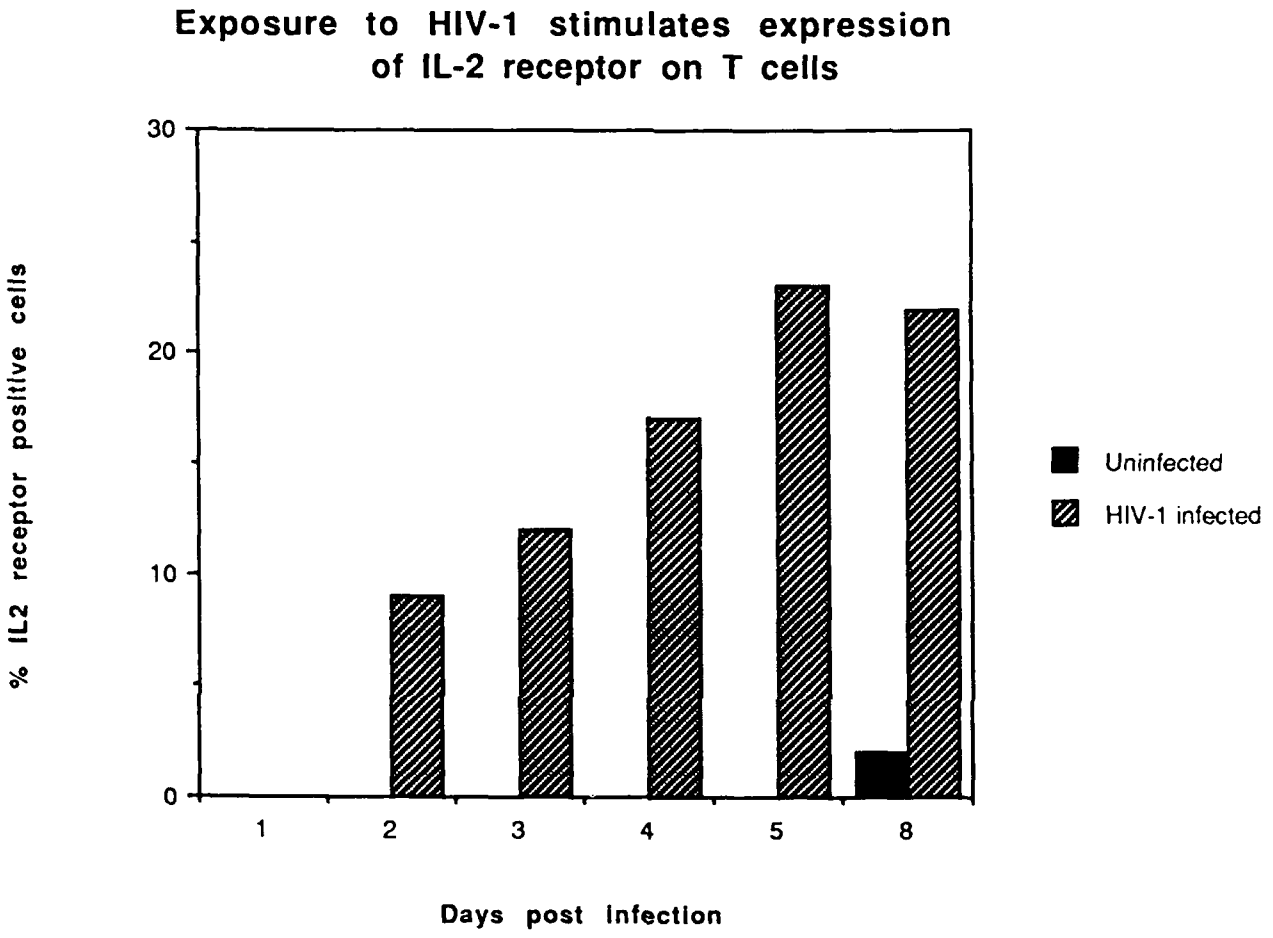

Exposure to HIV-1 stimulates expression of IL-2 receptor on T cells
% IL2 receptor positive cells
30
20
10
0
1
2
3
4
5
8
Days post infection
Uninfected
HIV-1 infected

FIGURE 4

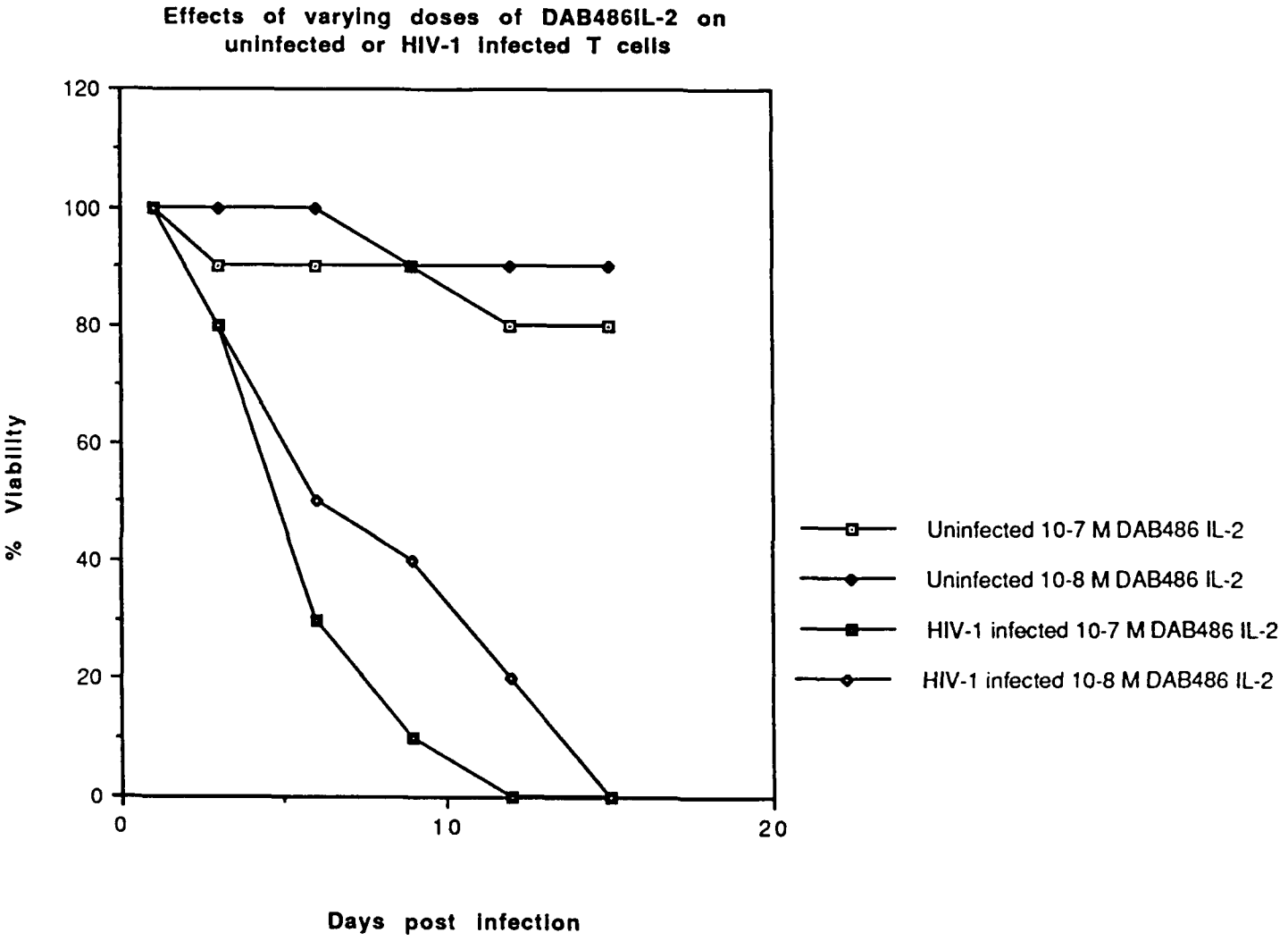

Effects of varying doses of DAB486IL-2 on uninfected or HIV-1 infected T cells
120
100
80
60
40
20
0
% Viability
0
10
20
Days post infection
Uninfected 10-7 M DAB486 IL-2
Uninfected 10-8 M DAB486 IL-2
HIV-1 infected 10-7 M DAB486 IL-2
HIV-1 infected 10-8 M DAB486 IL-2

FIGURE 5

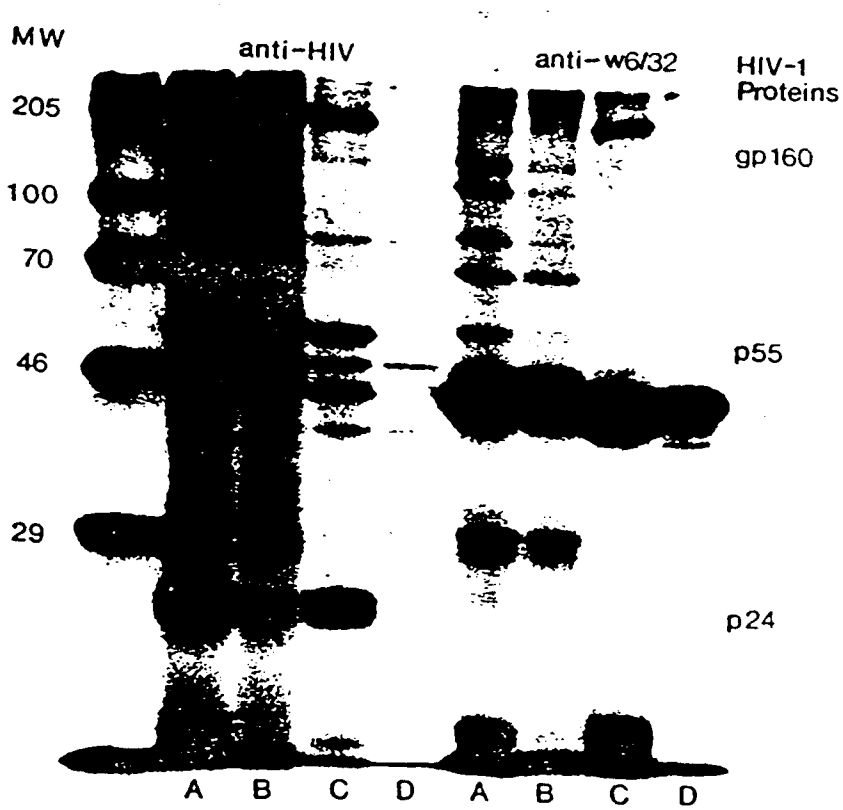
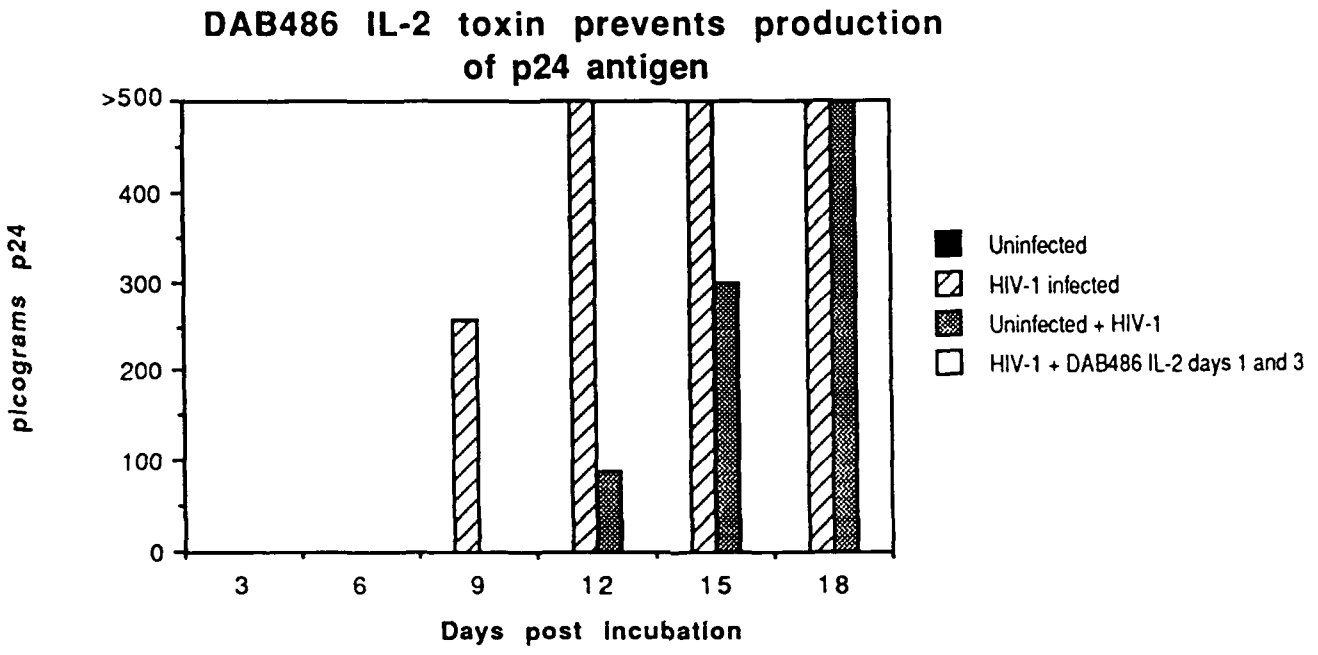

MW
anti-HIV
anti-w6/32
HIV-1
Proteins
205
100
70
46
29
gp160
p55
p24
A B C D A B C D

FIGURE 6


DAB486 IL-2 toxin prevents production of p24 antigen
picograms p24
>500
400
300
200
100
0
3
6
9
12
15
18
Days post incubation
Uninfected
HIV-1 infected
Uninfected + HIV-1
HIV-1 + DAB486 IL-2 days 1 and 3

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Heagy W, Laurance M, Cohen E, Finberg R. Neurohormones regulate T cell function. J Exp Med. 1990;171:1625-33.

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